THE GUT-BRAIN AXIS: HOW SELECT PROBIOTIC STRAIN ENHANCE COMPANION ANIMAL WELL-BEING

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OVERVIEW

Conclusive evidence has been accumulated that a strong neural and hormonal connection exists between the brain and the digestive system, referred to as the Gut-Brain Axis (GBA), in all mammals.

Based on extensive research, it is now known that the GBA serves as a cyclical, continuous communications infrastructure. This connection is supported through a network of 500 million neurons in the gastro-intestinal system, often referred to as the *"second brain"* by many researchers.

Recent research (Carabotti et al. 2015) indicates a symbiotic connection between gastro-intestinal microbiota and mental stress and anxiety as well as other disorders. The connection between the gastrointestinal system and human psychology was originally discovered in the 1880s. It has only been in recent years, thanks to advances in microbiology and exploration of the microbiota, that great strides have been made in understanding exactly how the GBA works across species.

It is known that a positive effect can be imparted on stress and anxiety – significant factors to ensuring overall companion animal well-being - through supplementation with specifically selected probiotics. (Ait-Belgnaoui et al.)

The benefits of reducing or eliminating microbial imbalances through probiotic usage in companion animal nutrition are many. The greatest benefit being the reduction of stress and anxiety, leading to a more harmonious relationship between the pet and pet owner. Secondary benefits include immune modulation, digestive support, controlling atopic dermatitis and curbing the prevalence of obesity.

The purpose of this paper is to explain in detail the Gut-Brain Access "Superhighway" – what it is, how it works, why it is important to pet well- being, and the important role strain-specific *Lactobacillus* Rosell-52 and *Bifidobacterium* Rosell-175 from Lallemand have in reducing stress and anxiety in pets.

Ultimately, it is about maintaining the close, familial bond between the pet and its owner/parent through the administration of probiotics.

THE GUT-BRAIN AXIS AND PET STRESS AND ANXIETY

The link between the intestinal tract and the nervous system and brain is directly related to animal well-being, and the impact of stress (Carabotti et al.). The brain and the microbes that inhabit pets' intestinal tracts continually communicate bi-directionally between the central nervous system (CNS), the enteric nervous system (ENS), the neuroendocrine system, and the immune system. This communication is all via the neuron-rich, hormonal GBA superhighway. The underlying support for this communication system involves neural, immune and endocrine pathways.

This network of neurons in the gut serves to integrate the outside world with the inside of a mammal. The GBA is capable of influencing the area of the brain responsible for memory and learning. This level of sophistication was first proven by Pavlov and his research with dogs salivating at the mere sight of the person who feeds them.

The signal sent from the gut to the brain may go through two main pathways: the humoral pathway, via hormones, or along the autonomic nervous pathway.

THE HUMORAL PATHWAY IN THE GUT-BRAIN AXIS

On the humoral pathway, the hypothalamus releases Corticotrophin Release Factor (CRF) in response to stress. The portal system carries CRF to the pituitary gland, which in turn secretes Adrenocorticotrophic Hormone (ACTH).

ACTH stimulates the adrenocortical gland, which produces glucocorticoid hormones, commonly known as cortisol – the stress hormone – in humans. High cortisol concentrations correlate with acute psychological stressors, suppressed immunity and affect inflammatory pathways.

Cortisol reacts systemically and leads to the opening of tight junctions – the space between epithelial cells in the intestine – and reduces intestinal protection.

Microbes can then enter the body and cause an inflammatory response. Inflammation stimulates release of additional cortisol, starting a perpetual cycle of inflammation, additional intestinal barrier loss, and weakened ability to fight off harmful intruders.

HPA coordinates adaptive responses against stress, including activation of memory and emotional centers in the brain. It is a major part of the neuroendocrine system that controls physiological response to stress, including elevation of two hallmark stress hormones, corticosterone and adrenocorticotrophic.

HPA regulates processes such as energy storage and expenditure, digestion, the immune system, mood and emotions.

THE AUTONOMIC NERVOUS PATHWAY IN THE GUT-BRAIN AXIS

In the autonomic nervous pathway, norepinephrine is released by the brain and from the postganglionic neurons of the sympathetic nervous system. Part of the autonomic nervous system, these neurons transmit the fight-or-flight response in each tissue. The animal's heart rate increases, the breathing intensifies, and blood flows towards the muscles to prepare for action.

Stress stimulates negative changes to the gastric system. Since blood flows towards muscle, there is less blood flow to the pet's stomach. A reduction of oxygen flow then triggers a lack of appetite and slowdown of digestion, causing intestinal motility and changes to the intestinal microflora. This imbalance often triggers diarrhea and abdominal discomfort.

Norepinephrine can provide a positive environment for viruses and bacteria by increasing their capacity to adhere to epithelial cells. This will increase their capture by dendritic cells, thus raising the ratio of antigen to lymphocytes, leading once again to increased inflammation.

When there is an inflammation of the intestinal sub-mucosal tissue, an afferent signal is sent to the brain through the vagus nerve. This signal of inflammation is perceived by the brain as a new, endogenous stressor. Added to the external stressor, it will lead to psychological, physiological and behavioral symptoms.

The Enteric Nervous System (ENS), an extensive network of neurons lining the gut, allows a bi-directional communication through blood vessels and nervous pathways through the vagus nerve. The vagus nerve comes directly from the brain to interface with parasympathetic control of the heart, lungs and digestive tract. If the vagus nerve is purposely severed to measure response, the effects of gut bacteria on brain biochemistry, stress response and behavior evaporate.

The intestine can send a message to the brain through neuroactive compounds such as serotonin. Gut bacteria manufacture about 95 percent of the body's supply of serotonin, which influences both mood and gastrointestinal activity. It goes systemically through blood circulation up to the brain. The gut microbiota is a major factor in this communication, as it metabolizes ingested food into tryptophan, gamma-aminobutyric acid (GABA), opioid-like compounds and other potentially neuroactive biochemicals.

Specialized intestinal endocrine cells, enterochromaffins, located in the gut produce large quantities of the neurotransmitter serotonin from tryptophan and histamine which may be responsible for the major effects on signaling to the brain.

Bacterial species can produce these neurotransmitters from tryptophan. Bacteria can modulate the release of cytokines

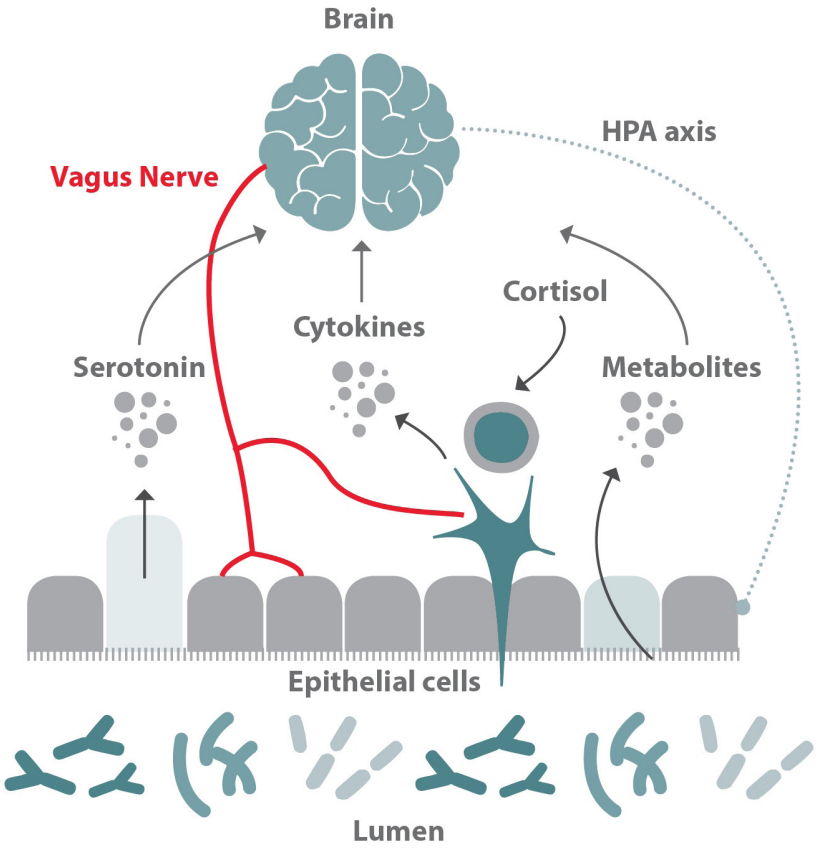


Figure 1:

The stress response in companion animals is mainly regulated and controlled by the Hypothalamus-Pituitary-Adrenal (HPA) axis.

from enteric immune cells. In turn, those cytokines can modulate the limbic system – the portion of the brain involved in emotion, learning and memory, again via the vagus nerve. Inflammatory cytokines disrupt brain neurochemistry and increase vulnerability to anxiety and depression.

Just as gut bacteria affect the brain, the brain can exert profound influences on the gut microbiome. The microbiome refers to all microorganisms in or on their host as well as their genetic material. There are approximately 10^{14} microorganisms in the microbiome, which is tenfold more cells than there are cells in the human body. Formation of the microbiome begins with exposure to the mother's vaginal microbiota and subsequently environmental microbes, and is essential to the early formation of the CNS and ENS.

Figure 2:

Insights into the gut-brain cross-talk have revealed a complex communication system that not only ensures the proper maintenance of gastrointestinal homeostasis, but is likely to have multiple effects on motivation and higher cognitive functions.

Its role is to monitor and integrate gut functions as well as to link emotional and cognitive centers of the brain with peripheral intestinal functions and mechanisms such as immune activation, intestinal permeability, enteric reflex and entero-endocrine signaling.

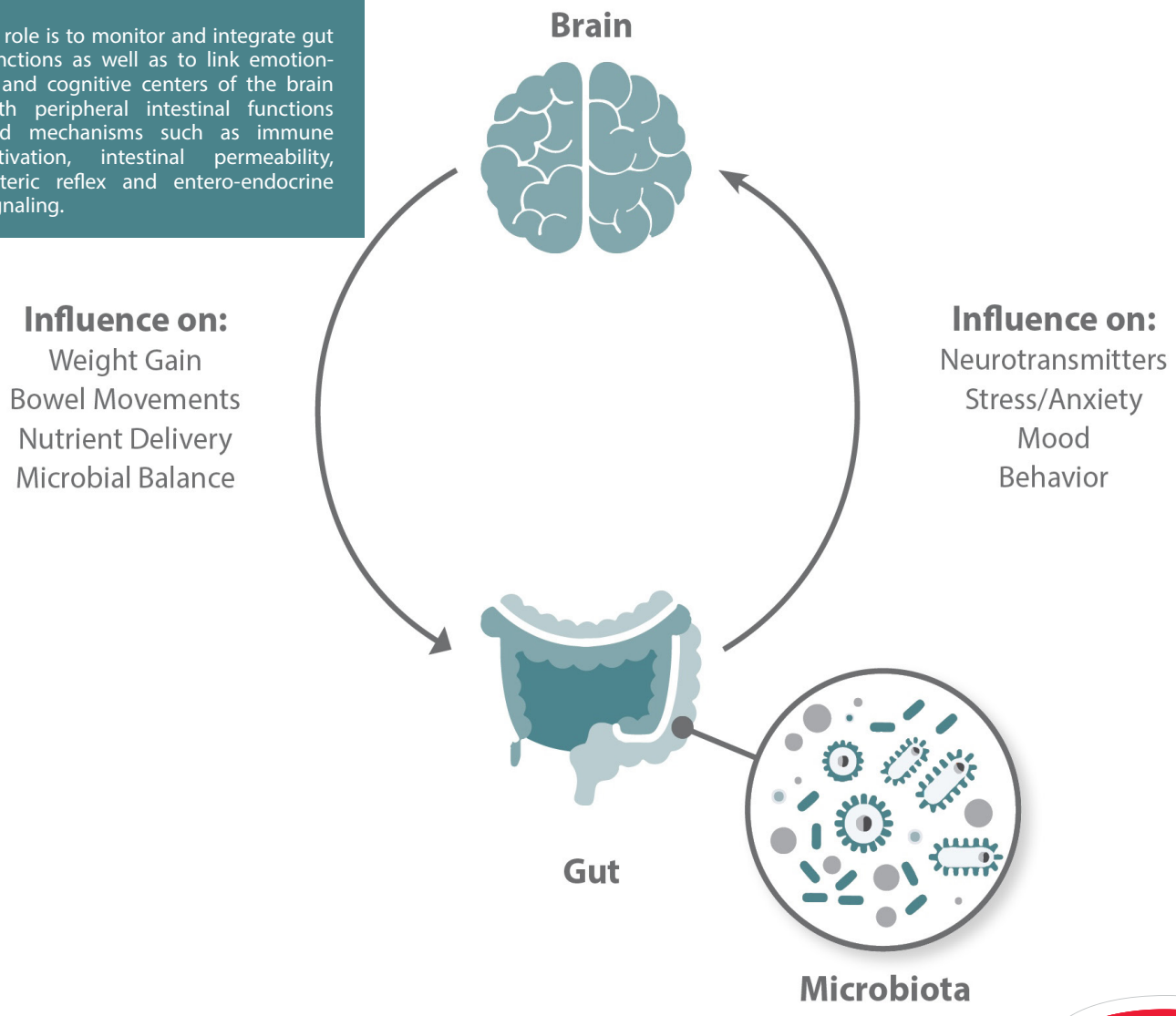
MICROBIAL IMBALANCE, STRESS AND BRAIN CELL DEATH

Anxiety and stress can cause an imbalance of intestinal microbiota, a process called dysbiosis. Dysbiosis can occur as a result of changes in diet, antibiotics, medications, or an infection. Dysbiosis can impact GBA, and thus result in cognitive issues and mood disorders.

Dysbiosis can cause a loss in bacterial diversity, leading to disruption of the microbiota and the production of negative neuroactive signals, decreased production of metabolites, and increased production of inflammatory cytokines.

All of these can send adverse messages to the brain which can result in apoptosis, or programmed cell death, and loss of neuroplasticity in the limbic regions of the brain.

Certain probiotics, when administered orally, have been shown to reduce inflammation and to reverse stress-induced apoptosis in the limbic system, as well as restore neuroplasticity and decrease anxiety.



LONG-TERM STRESS IMPACT

Stress can lead to a total breakdown between the emotional bond between the pet and its owner, and relinquishment to a shelter ensues.

CAUSES OF STRESS AND ANXIETY IN PETS

- Maternal and owner separation
- Fear of water/forced swimming
- Loud noise phobias including thunderstorms and fireworks
- Fear of strangers and unfamiliar surroundings
- Fear-related aggression towards them by animals or humans

SIGNS OF STRESS

- Hyper vigilance
- Aimless wandering
- Reluctance to take treats
- Excessive barking
- Panting without heat stress
- Excessive licking of lips and paws
- Listlessness and disinterest in exercise
- Aggression towards other pets and people

Physiological Response to Formal Anxiety Test

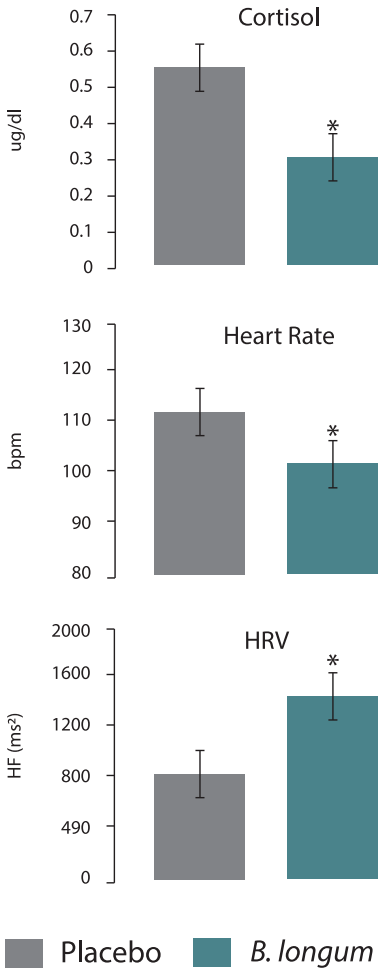


Figure 3:
A 2016 study on 24 dogs by Dr. Ragen T.S. McGowan, PhD, found a probiotic blend containing *Bifidobacterium longum* had an "anxiolytic effect on anxious dogs."

PROBIOTICS TAKE A CENTRAL ROLE

Probiotics are live organisms that confer beneficial effects on the recipient when delivered in adequate amounts. The most commonly used probiotic organisms include *Lactobacillus spp.* and *Bifidobacterium spp.* Additional bacteria used include *Bacillus spp* and *Enterocuccus spp* as well as the yeast, *Saccharomyces boulardii* (Barko et al.).

Various probiotic strains have demonstrated ability to re-balance the gut microflora, to relieve stress and anxiety, strengthen immunity and break the cyclic chain of inflammatory responses. Equilibrium of the gut microflora and the balance between positive and potentially negative bacteria is the objective to keep this system working, relieve environmental stresses and prevent dysbiosis.

The benefits of probiotic microorganisms are linked to their properties and metabolic activity in the host. Their modes of action are increasingly well understood.

In the gut, these effects could be divided between three levels:

• **Antioxidant Optimization:** Deactivate or eliminate potential microbes to prevent attachment and colonization.

• **Digestive Care:** Preserve the intestinal barrier's integrity and metabolism.

• **Immune Support:** Participation in the body's defenses by interacting with the immune system.

Research has found that adjusting the balance between beneficial and disease-causing bacteria in an animal's gut can alter its brain chemistry and lead it to become either bolder or more anxious. (Bercik et al.)

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THE SYNERGY BETWEEN BACTERIA AND YEASTS

The gut environment is composed of a diverse population of microbes, heavily skewed toward bacterial strains, which account for 98 percent of all microbes, compared to a much lower, but equally important population of yeast strains.

Gut bacteria regulate digestion and metabolism. They extract vitamins and other nutrients from food that is eaten, program the body's immune system, and build and maintain the gut wall, which protects the body from potentially harmful outside influences. Beneficial bacteria in the gut block harmful microbes and produce anti-microbial chemicals that defend the host against negative microorganisms.

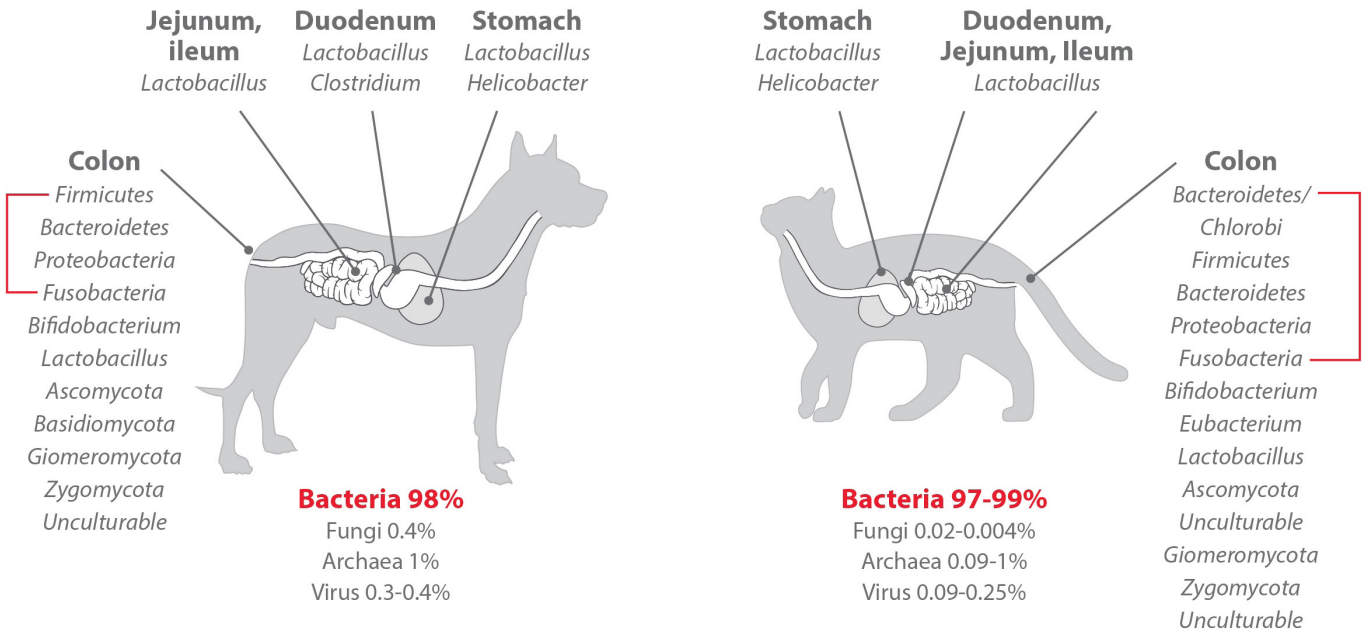

Figure 4:

All animals, dogs and cats included, harbor a vast and complex community of microorganisms, the so called Microbiota. This huge microbial community is present throughout the gastrointestinal tract and bacteria represents about the 98% of this community.

The predominant phyla in the colon of both dogs and cats are *Firmicutes, Bacteroidetes, Proteobacteria* and *Fusobacteria* but of course in different proportion. The Microbiota is a dynamic system and its composition within individual as well between individuals. It can be compared to a fingerprint: every individual has a unique one.

Most important among bacterial strains are *Firmicutes, Bacteriodetes, Proteobacteria and Fusobacteria* (Zhu et al.). *Firmicutes* and *Bacteriodetes* are predominant and represent 75 percent of the gut microbiota, on average, and both strains are very sensitive to change.

These bacteria, along with *Lactobacilli* and *Bifidobacteria*, deliver a synergistic effect with yeast, such as *Saccharomyces cerevisiae boulardii*. When combined, they have the ability to re-balance endogenous microflora in gut health.

This mixture of bacteria and yeast, the gut microbiota, is specific to each species and to each individual. Together, they play three important roles:

• **Metabolic:** Providing nutritional benefits through fermentation and metabolism.

• **Protective:** The driver of the host's immune system.

• **Structural:** Supporting healthy epithelium tissue that lines the cavities and surfaces of blood vessels and organs throughout the body.

MECHANISMS EXPLAINING EFFECTS OF PROBIOTICS ON ANXIETY AND DEPRESSION

1. Competitive exclusion of gut pathogens by probiotics – certain harmful gut microbes produce substances shown to induce anxiety and depression in animals;

2. Reduction of gut permeability through the barrier effect that prevents the entry of harmful gut microbes and antigens, reducing inflammation;

3. Decrease pro-inflammatory cytokines. A link has been drawn between depression and high levels of certain inflammatory markers;

4. Direct communication with the CNS via vagal sensory fiber. When inflammation occurs, a signal is sent to the brain via the vagus nerve. As inflammation decreases, the signal decreases.

OBSERVED EFFECTS OF PROBIOTICS ON PSYCHOLOGICAL DISTRESS

1. Both *Lactobacillus* Rosell-52 and *Bifidobacterium* Rosell-175 exert anti-inflammatory properties on human intestinal epithelial cells (Wallace et al.)

2. Both strains reduce intestinal permeability due to their barrier effect.

3. *Lactobacillus* Rosell-52 protects gut microflora against harmful bacteria. (Wine et al.)

Taken together, *Lactobacillus* Rosell-52 and *Bifidobacterium* Rosell-175 reduce the inflammation and neuro-inflammation caused by stress at the gut mucosa level and may explain the clinical effects this strain combination has on both physical and psychological symptoms of stress.

PROBIOTICS AND PREMIUM, "HUMANIZED" PET FOODS

Pet owners are not only looking for healthier, happier pets, but ones that *live longer.*

Probiotics in pet foods have been rising in demand as new research shows the increasing benefits of strain-specific probiotics to the pet's gut health, as well as influencing stress and anxiety.

Consumers are willing to pay for better pet nutrition. This has led to a rise in spending on nutritionally sound nutrition for pets. The global pet food market is an estimated $70 billion, with $24 billion of that spent in the United States alone.

RESEARCH

By 2017, 52 studies had been completed to evaluate the link between probiotic supplementation and the Gut-Brain Axis. Of those, 35 studies were in *vivo* in mice, while 17 studies in humans show the health of the gut influences the health of the brain, and vice versa. Some important examples are:

• In 2016, McGowan, Nestle Purina Research, studied *Bifidobacterium longum*, and found that exposure to a probiotic blend containing *B. longum* alleviated psychological stress in human volunteers and reduced anxiety-like behavior in rats. Studies have shown that this anxiolytic effect is achieved through the vagal pathway. In McGowan's own study in 24 dogs, they found that, "From both a behavioral and psychological standpoint, *B. longum* had an anxiolytic effect on anxious dogs."

In the same study, 90 percent of the dogs supplemented with *Bifidobacterium longum* showed improvement in day-to-day anxious behavior – reduction in barking, jumping, spinning and pacing when compared to a placebo.

Additionally, 80 percent of dogs showed a decrease in heart rate and an increase in heart rate variability, indicating a more positive response to the formal anxiety test when supplemented with *Bifidobacterium infanti.*

• Studies have shown that psychological stress suppresses beneficial bacteria. In a 2004 study by Bailey et al. published in the *Journal of Pediatric Gastroenterology*, infant monkeys whose mothers had been startled by loud noises during pregnancy had fewer *Lactobacilli* and *Bifidobacteria*.

• In 2004, Sudo et al. found that fecal transplanted *Bifidobacterium* infantis in mice returned HPA stress response to normal. In a situation of chronic stress, a vicious cycle of inflammation is initiated where cortisol released in the HPA stimulates mastocytes, or white blood cells, which play a part in inflammation during the process of healing. Most intestinal inflammation is stress induced.

• In 2011, Bercik and colleagues gave BALB/C mice, a strain that are typically timid and shy, a cocktail of antibiotics that dramatically changed the composition of their gut bacteria, and they became bold and adventurous. When the antibiotic regimen ended, the animals reverted to their usual, cautious selves, and their brain biochemistry returned to normal.

• Messaoudi et al. demonstrated that *Lactobacillus helveticus R0052* and *Bifidobacterium longum R0175* taken in combination for 30 days decreased scores for anxiety and depression related behaviors in human volunteers. *Bifidobacterium bifidum R0071* decreased stress-associated diarrhea-related symptoms and self-reported stress in a study on human academic stress by Culpepper et al. in 2015.

• Harmful bacteria can ramp up anxiety, and several studies have shown that beneficial bacteria can cause anxiety-prone mice to calm down. For example, a 2011 study by Bienenstock et al. showed mice fed *lactobacillus rhamnosus* showed an increase in the number of receptors for gamma-aminobutyric acid, or GABA, a neurotransmitter that mutes neuronal activity, keeping anxiety in check.

CONCLUSION

Probiotics are now on the cusp of becoming a standard treatment for stress and anxiety in companion animals.

Chronic stress occurs when the GBA superhighway streams continual, cyclical messaging that it is undergoing stress to the gut, causing dysbiosis and inflammation there. The inflamed gut then signals this reaction to stress back to the brain, kick-starting a new round in the gut-brain chronic stress cycle. Adverse messages sent to the brain can result in apoptosis, or programmed cell death, and loss of neuroplasticity in the limbic regions of the brain. Inflammatory cytokines can disrupt brain neurochemistry and increase vulnerability to anxiety and depression.

Lallemand has identified strain-specific *Lactbacillus* Rosell-52 and *Bifidobacterium* Rosell-175 as being synergistic and a key to breaking this chronic stress cycle by rebalancing gut microbia.

Until recently, probiotic supplements have not adequately explored the possibilities offered by the complementarity of both.

An expert in both yeast and bacteria production and applications, Lallemand Animal Nutrition has the know-how to develop specific probiotic formulations for the companion animal market.

Today, the company has selected a range of well documented bacteria strains for a new, holistic approach to digestive, physiological and psychological health and pet well-being.

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